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OXFORD UNIVERSITY PRESS | Journal of Analytical Toxicology

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Journal:	*Journal of Analytical Toxicology*
Manuscript ID	Draft
Manuscript Type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Salomone, Alberto; Centro Regionale Antidoping , Palamar, Joseph Bigiarini, Rachele Gerace, Enrico; Centro Regionale Antidoping, Di Corcia, Daniele Vincenti, Marco
Keywords:	Hair analysis, New Psychoactive Substances, NPS, new opioids, Fentanyl, U-47700, 4-ANPP

Detection of fentanyl analogs and synthetic opioids in real hair samples

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Keywords: Hair; new psychoactive substances; new opioids; fentanyl; U-47700; 4-ANPP

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Declaration of Interest: The authors declare no conflict of interest.

ABSTRACT

Novel synthetic opioids include various analogs of fentanyl and emerging non-fentanyl compounds with different chemical structures, such as AH-7921, MT-45, and U-47700. In recent years, these drugs have rapidly emerged on the drug market, and their abuse has been increasing worldwide. The motivations for use of these new compounds include their legal status, ready availability, low cost, users' curiosity or preference for their particular pharmacological properties, and the intention to avoid detection. Furthermore, more common drugs like heroin are now increasingly being replaced or cut with fentanyl or new designer opioids; thus, many drug users are unintentionally or unknowingly using synthetic fentanyl analogs. In this scenario, the detection of new psychoactive substances in hair can provide insight into their current diffusion among the population and social characteristics of these synthetic drug users. In this manuscript, we describe a simple, fast, specific and sensitive UHPLC-MS/MS method able to detect 13 synthetic opioids (including fentanyl analogs) and metabolites in hair samples. Furthermore, the method includes the detection of 4-anilino-N-phenethyl-piperidine (4-ANPP), which is considered both a precursor and a metabolite of several fentanyl analogs. The method was applied to 34 real hair samples collected in New York City from subjects who had reported past-year nonmedical opioid and/or heroin use. In total, 17 samples tested positive for at least one target analyte, with oxycodone (9 samples) and tramadol (8 samples) being the most common. Among these, the method was able to quantify furanyl fentanyl and fentanyl in the pg/mg range in two samples. Simultaneously, the molecule 4-ANPP was also detected, showing for the first time that this compound can be selected as a marker of fentanyl analog use via hair testing. In conclusion, this study confirmed the increasing diffusion of new synthetic opioids and "fentalogs" with high potency among nonmedical opioid users.

INTRODUCTION

For many years, the spectrum of illegal psychoactive drugs amounted to relatively few substances, commonly referred to as common/traditional drugs of abuse. More recently, a large upsurge of new psychoactive substances (NPS) has been observed (1) . Although most NPS fall into the classes of synthetic cannabinoids and designer cathinones (1), novel synthetic opioids (NSO) have also appeared recently on the drug market with a progressive increase in their consumption (2) Many of the NSO are derivatives of a well-known pharmaceutically used substance, fentanyl. However, new synthetic opioids such as AH-7921, MT-45, and U-47700, have chemical structures distinct from any known therapeutic or recreational drug. These substances are often sold as 'research chemicals' mainly through internet shops and used as substitutes of controlled opioids, but they are also increasingly sold under the guise of heroin posing a high risk of severe intoxication to unaware users (3). The motivations for the use of these new drugs include their legal status, availability, low cost, user curiosity or preference for their particular pharmacological properties, and the intention to avoid detection (2). Furthermore, of major public health concern is that common drugs like heroin are now commonly replaced or cut with new designer opioids (4,5). While heroin is the most common drug to be adulterated with these novel opioids, these compounds have been detected in cocaine, methamphetamine, ketamine, ecstasy, and counterfeit Xanax® and Percocet® pills (6-9). Therefore, many drug users are unintentionally or unknowingly using synthetic fentanyl analogs (10). Since these compounds on the 'street' market vary in composition and potency, their use may lead to severe intoxications and overdose fatalities—particularly among opioid-naïve individuals in which overdoses are not as easily reversed with naloxone (8). Several cases of intoxications and deaths related to the uptake of NSO have been reported to the United Nations Office on Drugs and Crime (UNODC) and have been summarized in the Global SMART Update on Fentanyl analogues (11). Fentanyl has been particularly problematic in the US, in which in 2016, was detected in over half (56%) of opioid-related deaths, and its analogs were detected in

14% (12). As a consequence of increasing prevalence and emergence of new compounds, forensic and clinical laboratories worldwide are continuously requested to update their analytical procedures for the identification and quantification of these new drugs in various biological matrices. Few preliminary methods have been published for the detection in conventional matrices such as urine (13) and blood (13,14), and more generally in biological specimens (15). Recent review papers have summarized the cases of fatal overdoses and the analytical approaches used for the investigation of such cases (16, 17).

To the best of our knowledge, comprehensive methods for testing fentanyl and/or its analogs in hair have not been published yet. Furthermore, the detection of new synthetic opioids in hair samples can serve as a practical means to provide preliminary information on black market penetration of these analogs in specific territories and populations, similar to how recent testing for synthetic cannabinoids and synthetic cathinones has been utilized (18-25). The keratin matrix incorporates the parent NPS consumed over extended time periods, providing access to a much wider diagnostic window than urine and saliva (26). This feature, combined with the analytical performances of the last generation ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) instruments, allows researchers to obtain significant information about past exposure, even a single intake, of any targeted NPS, with older periods of use corresponding to the hair segments more distant from the hair root (27,28).

In the present study, we focused on the specific population of electronic dance music (EDM) nightclub and festival attendees in New York City (NYC), as a high-risk population for both intentional and unintentional exposure to common drugs (29) and various NPS (30,31). Inclusion of both common opioids/opiates and novel opioids in our screening allows us to distinguish different consumption patterns, including co-use, occasional NSO intake, and frequent use. To our knowledge, this is the first study to examine hair results of new opioids use in a selected population.

EXPERIMENTAL

Sample preparation

Briefly, about 25 mg of hair was twice-washed with dichloromethane and then methanol (2 mL, vortex mixed for 3 min). After complete removal of solvent washes, the hair was dried at room temperature by a gentle nitrogen flow and subsequently grinding with a ball mill (Precellys 24, Bertin Instruments, Montigny-le-Bretonneux, France). Hair samples were fortified with 2.5 µL of an internal standards mixture yielding a final concentration of 0.01 ng/mg. After the addition of 1 mL of methanol, the samples were incubated at 55 °C for 15 h without stirring. Lastly, the organic phase was collected and an aliquot of 2 µL was directly injected into the UHPLC-MS/MS system. Whenever the real samples concentrations were found to exceed the highest calibration point, the final extracts were diluted with methanol and re-injected into the system.

Instrumentation

All analyses were performed using a Shimadzu LC-30A Series system (Shimadzu, Duisburg, Germany), interfaced to an API 5500 triple quadrupole mass spectrometer (Sciex, Darmstadt, Germany) equipped with an electrospray Turbo Ion source operating in positive-ion mode. A CORTECS UPLC C18 plus column 100 mm × 2.1 mm, 1.6 µm (Waters Corporation, Italy), protected by a C18 waters VanGuard pre-column, was used for the target analytes separation.. The column oven was maintained at 45°C and the elution solvents were water/formic acid 5 mM (solvent A) and acetonitrile plus formic acid 5 mM (solvent B). After an initial isocratic elution at 95% A for 0.5 min, the mobile phase composition was varied by a linear gradient (A:B; v/v) from 95:5 to 50:50 in 3.5 min; then isocratic elution at 50% B was maintained for 0.5 min. The flow rate was 0.5 mL/min and the total run time was 6 min including re-equilibration at the initial conditions before each injection. MS/MS detection was executed in the selected reaction monitoring (SRM)

mode. In order to establish appropriate SRM conditions, each analyte was individually infused into the electrospray ionization (ESI) capillary while the declustering potential (DP) was adjusted to maximize the intensity of the protonated molecular species $[M+H]^+$. The collision energy (CE) was set so as to preserve approximately 10% of precursor ion and the cell exit potentials (CXP) were also optimized. The SRM transitions were monitored during a time window of ±17.5 s around the expected retention time, and the cycle time of the SRM program was 0.100 s. Optimal signals were obtained using a source block temperature of 600°C and an ion-spray voltage of 1900 V. Gas pressures were set as follows: curtain gas 35 psi, ion source gas (1) 45 psi and ion source gas (2) 50 psi. SRM transitions and potentials for the analytes and internal standards are presented in Table 1.

Method validation

The following parameters were investigated: selectivity, specificity, linearity range, detection and quantification limits (LOD and LOQ), intra-assay precision and accuracy. Carry-over effect, recovery and matrix effects were also investigated. One qualifying SRM transition was monitored, in addition to the primary fragmentation (see Table 1). Variations of mass transitions intensities were considered acceptable within ±20%, with respect to the corresponding control. Specificity was determined on five blank head hair samples. The signal-to-noise ratio (S/N) was measured on the less intense mass transition at the expected analyte retention time. A S/N < 3 was considered satisfactory in order to verify the method's specificity.

The calibration process was conducted with an optimized procedure, requiring the preparation of three replicates of the calibration curves spiked with the working solution at six concentration levels (1, 2, 5, 10, 20, and 50 pg/mg and 10, 20, 50, 100, 200, 500 pg/mg for Oxycodone), for three consecutive days for a total of nine calibration curves. The data from each specific calibration curve were quantified using a calibration curve obtained in a different day, allowing to manage each set of data as independent. Therefore, 9 samples (from 9 different batches) per each calibration level were employed to estimate the previously cited validation parameters. The calibration was completed by internal standardization. The linear calibration parameters were evaluated using the least squares

regression method; several significance tests were performed to evaluate linearity, including lack-of-fit test and analysis of variance (ANOVA) (see Table 2). Determination coefficient (R^2), adjusted determination coefficient (Adj R^2), relative standard deviation of the slope, normality of the standardized residuals, and deviation from back-calculated concentrations were also evaluated. The LODs were estimated using the Hubaux-Vox approach (32), and the LOQs were then approximated as 3 times the LOD values. In-house developed spreadsheets were employed to evaluate these parameters and perform their relative significance tests. For all analytes, intra- and inter-day precision (expressed as percent variation coefficient, CV%) and accuracy (expressed as bias %) were evaluated at three concentration levels. The samples prepared for the evaluation of linearity, LOD and LOQ parameters were used, thus consisting of nine replicates (prepared and analyzed in three different days) of blank hair samples spiked with the standard solutions at the lowest calibration point (close to LOQ values), at intermediate calibration level, and the highest calibration point. Intra-day and inter-day precision parameters were considered satisfactory when CV% values turned below 15%. Satisfactory accuracy (in terms of bias %) was achieved when the experimentally determined average concentration lied within ±20% from the expected value. Extraction recoveries were determined by comparing the responses obtained from samples (five replicates) initially spiked with the analytes at a concentration of 20 pg/mg (200 pg/mg for Oxycodone) and subsequently extracted and processed as usual, with the responses of blank samples in which the analytes were added at the same concentration after the extraction step. The matrix effect was calculated, by comparing the peak area ratio between analyte and ISTD obtained from spiked hair samples, with the corresponding ratio obtained from a pure methanol solution, at the same concentrations. In this case, the matrix effect is expected to be partly compensated by a well-matched internal standard, i.e. the isotopically-marked analyte, whenever possible, or the one having the closest RT to the analyte, so as to undergo similar interference from the matrix. The matrix effect was calculated as the mean value obtained from five different hair sources. The percent difference represented either matrix suppression (values below 100%) or matrix

enhancement (values above 100%). The possible presence of carry-over effects was evaluated by injecting an alternate sequence of five blank head hair samples and five blank head hair samples spiked with all analytes at the highest concentration point of the calibration curve (50 pg/mg and 500 for Oxycodone). To ensure the absence of any carry-over effect, the signal to noise ratio had to be lower than 3 for each monitored transition.

Analysis of real samples

Subjects were eligible if they identified as age 18-40 and were about to attend the randomly selected party in NYC. After providing informed consent, subjects were surveyed about use of dozens of drugs including 18 common and novel opioids (33). At the end of the survey, participants were asked if they were willing to donate a hair sample to be analyzed at a future date. Collection was conducted from July through September of 2017. This study focuses on the participants who reported any past-year nonmedical opioid or heroin use and provided a useable hair sample for analysis. If the participant agreed, the trained recruiter collected the sample by cutting a small lock of hair (~100 hairs) from as close to the participant's scalp as possible. Hair was cut with a clean scissor (wiped with an alcohol-wipe after each use), folded up in a piece of tin foil, and stored at room temperature in a small envelope labeled with the participant's anonymous study ID number (34). A total of 34 real hair samples was collected. This study was approved by the New York University Langone Medical Center Institutional Review Board.

Results and discussion

The optimized UHPLC–MS/MS method allowed the simultaneous determination of 13 fentanyl analogs and novel synthetic opioids in hair samples, plus three internal standards. The whole chromatographic run, comprehensive of the time required for column re-equilibration before the following injection, was completed in 6 min. Retention times ranged between 2.0 min (oxycodone) and 3.70 min (sufentanil). Tramadol produced only one product ion (m/z 58.1) Figure 1 shows the

SRM chromatograms recorded from a blank hair spiked with all analytes at 5 pg/mg concentration (50 pg/mg for Oxycodone).

Validation

All the validation results are reported in Table 2. No carry-over effect was observed under the conditions described in the experimental section. Selectivity and specificity tests proved successful, i.e., SRM chromatograms from negative head hair samples showed no interfering signals at the retention time where the analytes were expected to elute. The trend of the variance associated to the tested calibration points (i.e. homoscedasticity vs. heteroscedasticity) was evaluated. A weighting factor equal to ($1/x^2$), where x represents the concentration of the calibration point, was therefore adopted for all analytes because the data points distribution turned heteroscedastic. The lack-of-fit and ANOVA tests suggested a linear regression model for all analytes. Table 2a reports a summary of the observed results, including the Adj R^2 values of the regression models. LOD and LOQ values estimated by Hubaux-Vos' methodology are reported, too. In particular, LOD values were in the range 0.1-0.3 pg/mg for all analytes, with the exception of oxycodone which in turn resulted to be 1.5 pg/mg. LOQ values lied between 0.3-0.9 pg/mg and 1.5 pg/mg, respectively. The calculated LODs were experimentally confirmed with one blank hair sample spiked at the estimated LODs concentrations. Extraction recoveries were mostly close to 100% and always in the interval 100±30%, as estimated from samples spiked at 20 pg/mg concentration (200 pg/mg for Oxycodone). The effect of the real hair matrix appeared to be significant for some of the analytes tested, particularly when the absolute peak areas are considered (ion suppression for oxycodone and tramadol). While, after peak area correction using the internal standard (Table 2b), there are not significant ion suppression phenomenon, nor ion enhancement. The variability among different hair samples turned out acceptable (CV% <25%) for all the analytes, provided that the results are normalized using the ISTD. Furthermore, the good linearity observed in the calibration plots

supports the observation of constant percent matrix effect, which in fact does not depend on the analytes' concentration. Intraday precision and accuracy were satisfactory for all analytes at all calibration level (1, 10 and 50 pg/mg and 10, 100, 500 for Oxycodone), while modest deviation was observed in the evaluation of intraday accuracy for remifentanil and sufentanil.

Analysis of real samples

A total of 34 samples was considered to test the applicability of the developed method. Among these, 17 (50%) tested positive for at least one opioid compound. The molecules detected were oxycodone (9 samples, range of concentrations: 13-780 pg/mg), tramadol (8 samples, range 2.0-3700 pg/mg), hydrocodone (4 samples, range 13-71 pg/mg), fentanyl (2 samples, respectively 3 and 6 pg/mg), furanyl fentanyl (1 sample, 44 pg/mg), and 4-ANPP (2 samples, respectively 2 and 1 pg/mg). The other compounds included in the panel of target analytes were never detected in the selected samples. Fentanyl and furanyl fentanyl were measured in the level of low pg/mg, suggesting low rate of incorporation into the keratin matrix or more likely the exposure to low quantities of the drug. Like other classes of NPS (35), more data about the detection of NSO in hair are needed before any interpretation of analytical findings can be given. All samples were also tested for common opiates (i.e. heroin) with a previously published method for traditional drugs of abuse (36). Remarkably, only the two samples positive to fentanyl also showed a high concentrations of heroin metabolites. Unfortunately, because of the low quantity of hair available, it was not possible to perform a segmental analysis to discriminate if fentanyl and heroin had been taken in the same period. Therefore, it is hard to speculate if the fentanyl was taken alone or as a heroin cutting agent. However, one of the two subjects whose hair tested positive for fentanyl reported known past-year use of fentanyl when surveyed, and the other denied ever using, so in this case it is likely the NSO were consumed unknowingly. The metabolite norfentanil could not be detected in the two samples which were positive to fentanyl at low concentrations. It is well known that metabolites are usually incorporated in hair samples at lower rate than the parent compound, so likely these two cases presented norfentanyl at too low levels to be detected by our method. On the

other hand, we were able to detect 4-anilino-N-phenethyl-piperidine (4-ANPP) in one sample positive to fentanyl and fluorofentanyl, with comparable concentrations. The 4-ANPP is considered both a precursor and a metabolite of several fentanyl analogs (14, 37-39), therefore its detection in biological samples can work as a promising marker of some fentanyl analogs intake, even when the (newly introduced) drug is not included in the panel of the target analytes.

CONCLUSIONS

The present study demonstrated that 13 new synthetic opioids can be determined in the keratin matrix with high sensitivity and specificity, allowing wide-range monitoring of drug intake over extended periods of time. In the future, more compounds pharmacologically related--either analogs of fentanyl or with different chemical structures--should be added to the panel of the target analytes. As a matter of fact, the situation of the black market is unpredictable; thus, a comprehensive screening method for NSO is needed for a realistic picture of use of such compounds in specific populations and at different times. In this scenario, the use of 4-ANPP as marker of the fentanyl analogs looks extremely promising in hair.

Furthermore, large NSO screening panels using hair testing and taking into account the timely fluctuation of the market situation can finally be applied to other individuals under periodic control (e.g., in workplace control or driving re-licensing) to check how frequently these compounds are used, either knowingly or unknowingly.

ACKNOWLEDGMENTS

Research reported in this publication was supported by the National Institute on Drug Abuse of the National Institutes of Health under Award Number K01DA038800. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Table 1. SRM transitions and corresponding potentials for the target compounds and internal standard detection

	Compound	RT (min)	Precursor Ion $[M+H]^+$	DP (V)	Target			Qualifier 1			Qualifier 2		
					Fragment	CE (V)	CXP (V)	Fragment	CE (V)	CXP (V)	Fragment	CE (V)	CXP (V)
1	Oxycodone	2.1	316.0	70	241.1	38	11	298.1	25	14	256.1	34	11
2	Hydrocodone	2.1	300.0	76	199.1	42	9	171.1	51	7	128.0	74	15
3	Norfentanyl	2.4	233.0	50	84.1	24	9	150.1	22	14	55.0	50	8
4	Tramadol	2.6	264.1	35	58.1	46	11	-	-	-	-	-	-
5	Remifentanil	2.8	377.1	43	317.2	22	14	228.0	27	8	116.1	37	14
6	Acetyl-Fentanyl	3.0	323.0	73	188.2	38	15	105.0	38	14	77.0	86	10
7	Alfentanil	3.3	417.0	52	268.3	24	8	197.2	35	9	165.0	47	16
8	U-47700	3.3	330.9	47	286.1	24	11	-	-	-	206.1	34	8
			328.9	47	-	-	-	204.1	36	7	-	-	-
9	Fentanyl	3.3	337.1	57	188.2	32	6	105.0	49	5	132.1	42	6
10	4-ANPP	3.3	281.0	72	188.2	24	8	105.0	41	12	103.0	63	16
11	Furanyl-Fentanyl	3.4	375.0	55	188.2	28	14	105.0	52	13	103.0	82	16
12	Carfentanil	3.5	395.0	50	335.2	25	11	246.1	34	9	113.0	34	6
13	Sufentanil	3.7	387.0	43	238.1	26	10	355.2	26	15	111.0	46	12
IS1	Oxycodone -d_6	2.0	322.0	70	247.1	38	11	-	-	-	-	-	-
IS2	Norfentanyl –d_5	2.4	238.0	50	84.1	24	9	-	-	-	-	-	-
IS3	Fentanyl –d_5	3.3	342.0	57	188.2	32	6	-	-	-	-	-	-

DP, Declustering potential; CE, Collision Energy; CXP, Cell Exit Potential.

Table 2a. Range of calibration, linearity, LODs and LOQs values, linearity tests over the calibration interval for all analytes

	Compound	Linearity Range (pg/mg)	Internal Standard (IS)	Linearity (Adj R^2)	LOD (pg/mg)	LOQ[a] (pg/mg)	Lack-of-Fit (F_{crit} = 2.37)	ANOVA test (F_{tab}=3.69)
1	Oxycodone	10-500	Oxycodone -d_6	0.9999	1.5	4.5	0.08	0.06
2	Hydrocodone	1-50	Fentanyl –d_5	0.9994	0.1	0.3	0.27	0.37
3	Norfentanyl	1-50	Norfentanyl –d_5	0.9995	0.1	0.3	0.37	0.18
4	Tramadol	1-50	Norfentanyl –d_5	0.9996	0.1	0.3	0.35	0.20
5	Remifentanil	1-50	Fentanyl –d_5	0.9968	0.3	0.9	1.93	0.70
6	Acetyl-Fentanyl	1-50	Fentanyl –d_5	0.9980	0.2	0.6	1.57	0.34
7	Alfentanil	1-50	Fentanyl –d_5	0.9992	0.1	0.3	0.65	0.21
8	U-47700	1-50	Norfentanyl –d_5	0.9998	0.1	0.3	0.09	0.28
9	Fentanyl	1-50	Fentanyl –d_5	0.9993	0.1	0.3	0.53	0.27
10	4-ANPP	1-50	Fentanyl –d_5	0.9994	0.1	0.3	0.48	0.21
11	Furanyl-Fentanyl	1-50	Fentanyl –d_5	0.9991	0.1	0.3	0.59	0.41
12	Carfentanil	1-50	Fentanyl –d_5	0.9983	0.2	0.6	0.76	0.72
13	Sufentanil	1-50	Fentanyl –d_5	0.9961	0.3	0.9	1.46	0.90

[a] calculated LOQ

Table 2b. Precision, accuracy, recovery and matrix effect for all analytes

	Compound	Intraday precision (CV%)			Interday precision (CV%)			Accuracy (bias%)			Recovery[a] (%)	Matrix effect[b]		Matrix effect[c]	
		1 pg/mg	10 pg/mg	50 pg/mg	1 pg/mg	10 pg/mg	50 pg/mg	1 pg/mg	10 pg/mg	50 pg/mg		Mean (±%)	CV (%)	Mean (±%)	CV (%)
1	Oxycodone	10	4	2	9	9	3	9	1	-0.2	95	-31	33	3	18
2	Hydrocodone	14	9	5	7	12	8	15	8	1	85	11	29	9	6
3	Norfentanyl	6	3	3	12	10	2	6	-6	-0.3	93	5	12	4	0.5
4	Tramadol	4	4	1	10	7	4	2	-5	-0.3	98	-30	16	10	7
5	Remifentanil	14	4	2	15	11	16	-19	-7	-1	71	4	21	5	11
6	Acetyl-Fentanyl	3	2	3	14	5	4	6	-3	-1	91	-8	13	-3	13
7	Alfentanil	4	4	3	14	13	1	-13	-5	-0.6	99	-4	10	9	2
8	U-47700	10	2	2	11	3	13	16	2	-0.2	112	3	20	8	7
9	Fentanyl	3	3	3	14	5	7	-13	-3	-0.6	96	4	31	10	11
10	4-ANPP	7	4	2	13	10	4	10	-8	-0.2	88	-3	22	3	22
11	Furanyl-Fentanyl	7	2	3	12	7	12	17	-4	-1	96	5	28	10	17
12	Carfentanil	6	3	3	15	14	8	-6	-3	-1	96	-9	5	5	0.5
13	Sufentanil	15	5	4	21	14	13	-19	-5	-2	92	-14	10	2	3

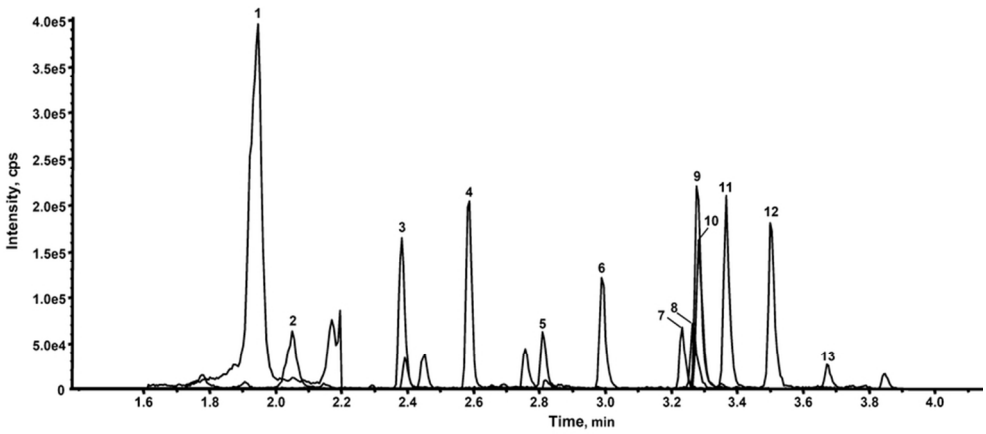
[a] Recovery evaluated at 20 pg/mg for all analytes except for Oxycodone tested at 200 pg/mg
[b] Matrix effect was evaluated using five different sources of hair (2 pg/mg for all analytes except for Oxycodone tested at 20 pg/mg)
[c] Matrix effect was evaluated on the same sources of hair, but in this case the effect was compensated by the use of ISTD
CV%, percent variation coefficient

Table 3. Results from samples which tested positive for at least one NSO (concentrations are given in pg/mg)

Subject		Detected analytes						Heroin metabolites		
Case	**Length (cm)**	**Fentanyl**	**Furanyl fentanyl**	**4-ANPP**	**Oxycodone**	**Hydrocodone**	**Tramadol**	**Morphine**	**6-AM**	**Codeine**
1	3.5	3	44	3	-	-	-	8700	>10000	580
2	7.5	6	-	1	-	-	-	260	3060	59
3	2.0	-	-	-	240	-	4	-	-	-
4	1.5	-	-	-	780	-	-	-	-	-
5	3.5	-	-	-	-	-	530	-	-	-
6	2.0	-	-	-	60	-	-	-	-	-
7	2.0	-	-	-	-	13	-	-	-	-
8	12.0	-	-	-	110	-	14	-	-	-
9	8.0	-	-	-	52	71	-	-	-	-
10	2.5	-	-	-	95	-	-	-	-	-
11	12.0	-	-	-	-	-	33	-	-	-
12	2.0	-	-	-	-	48	-	-	-	-
13	1.0	-	-	-	71	-	2	-	-	-
14	4.0	-	-	-	-	-	110	-	-	-
15	12.0	-	-	-	-	-	3700	-	-	-
16	6.0	-	-	-	13	-	22	-	-	-
17	5.0	-	-	-	13	48	-	-	-	-
Positive findings		**2**	**1**	**2**	**9**	**4**	**8**	**2**	**2**	**2**

Note. All samples were collected from participants' heads. 6-AM: 6-acetylmorphine.

Figure 1. SRM chromatograms recorded from blank hair sample spiked with all analytes at 5 pg/mg concentration (50 pg/mg for Oxycodone). For each analyte, labelled by the progressive number assigned in Table 1, only the target transition is shown.



81x37mm (300 x 300 DPI)